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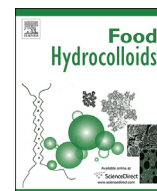
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Formation kinetics and rheology of alginate fluid gels produced by in-situ calcium release



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ABSTRACT

The formation and properties of alginate fluid gels produced using in-situ calcium release and a defined shear field are investigated.

Results show that, while the rate of particle growth during formation increases significantly upon lowering the shear rate, the rate of inter-particle interaction post-production remains unaffected by the magnitude of shear.

The longer polymer chain allows a greater number of possible sites for calcium crosslinking per chain which enhances the formation of a percolating network and increases the number of rheologically-effective network crosslinks. As a result, high M_w alginate fluid gels exhibit faster gelation kinetics and greater viscosities than those of low M_w alginate fluid gels.

Particle stiffness increases with increasing CaCO_3 concentration until a critical value is reached. Quiescent gels display the same CaCO_3 critical value, suggesting that the number of crosslinks within an individual fluid gel particle is equivalent to that of their counterpart quiescent gel. This is due to the fast coil-dimer transition which, under the applied shear field, leads to kinetically trapped fluid gel structures. However, their textural response differs from that of a quiescent gel which is likely caused by the bridging between fluid gel particles.

In addition to advancing the understanding of the production of fluid gels from alginates, this work shows for the first time how the ordering kinetics and the resulting particle properties can be manipulated by the choice of alginate M_w and CaCO_3 concentration, potentially allowing the design and delivery of specific structures with desirable attributes.

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1. Introduction

Alginates are linear polyuronic polysaccharide extracted from brown seaweed (Phaeophyceae), consisting of (1-4)-linked blocks of poly- β -D-mannuronic acid (M) and poly- α -L-guluronic acid (G) residues at different proportions and with different sequential occurrence (Draget, Skjåk-Bræk, & Stokke, 2006; Stokke et al., 2000).

Quiescent gelation of alginates with different M/G ratios has been extensively studied, where alginates with high content of guluronate have been shown to form strong, brittle gels, whereas alginates rich in mannuronate residues give softer and more elastic gels (Draget, Skjåk-Bræk, & Smidsrød, 1994; Matsumoto et al., 1992; Smidsrød, 1974). In that regard, recent molecular model investigations have demonstrated that calcium ions preferentially bind to the G-blocks which are stiffer and possess a more extended

chain conformation compared to the M-blocks due to a greater hindered rotation around the glycosidic linkages (Braccini, Grasso, & Pérez, 1999; Braccini & Pérez, 2001). The effects of both calcium and alginate M/G ratio on the rheological behaviour of alginate gels are discussed in great detail in the work of Mitchell and Blanshard (1976).

However, little attention has been paid to the influence of polymer molecular weight (M_w) on the time dependent gelation mechanism of alginate gels and, to our knowledge, no study has reported the effect of polymer M_w and Calcium on the formation of suspensions of alginate microgelled particles (i.e. fluid gels). Thus, the aim of the present work is to investigate the structural events dictating the formation of such particulate structures and the different properties that emerge from varying either processing conditions or the material (alginate M_w and calcium concentration).

Fluid gels are formed by application of a sufficiently energetic flow field to the polymer solution whilst undergoing conformational transition and consequent aggregation (Norton, Jarvis, & Foster, 1999; Norton, Smith, Frith, & Foster, 2000). In the case of

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alginates, the production of microparticles is particularly complicated due to the fact that the ordering process is not controlled by temperature and it occurs over the millisecond to seconds time scale (Goodall & Norton, 1987). A new developed technique employing a Pin-stirrer device has been recently used to produce alginate fluid gels. This technique involves the injection of a soluble form of Calcium (e.g. CaCl_2) near the impeller of the Pin, in the high energy dissipation zone, so that the alginate and calcium streams are quickly mixed inside the stirred vessel, thereby preventing large particles to form (Fernández Farrés, Douaire, & Norton, 2013).

By directly introducing an active form of calcium into the alginate solution and under shear, the rapid crosslinking reaction between the carboxyl groups of the polymer chain and the cations leads to the formation of anisotropic gelled particles with broad size distributions. This is due to differences in the diffusion rate of Calcium throughout the system, that is, the diffusion rate of Calcium through the gelled matrix is slower than the diffusion rate of Calcium through the aqueous continuous phase.

Poor control over the gelation kinetics characteristic to such diffusion-controlled methods can be overcome by gradually releasing the crosslinking ion from a less soluble form of Calcium (e.g. CaCO_3 , PHPO_4) triggered by the reaction with protons from the hydrolysis of a glucono- δ -lactone (GDL). This method (internal gelation) as previously described by (Draget, Østgaard, & Smidsrød, 1990; Mitchell & Blanshard, 1976) has been employed in the present study because it allowed the rate of ordering transition to be matched to the time scale of the imposed shear field. This shear field required to form fluid gels was provided by the rheometer, which enabled manipulation of the intensity of applied shear rate and monitoring of the viscoelastic response during processing. By using the rheometer in combination with internal gelation method the kinetics of fluid gel formation could be evaluated.

In this work, alginates with different M_w and equivalent G/M were used to produce a range of fluid gels. The structural changes occurring during fluid gel formation due to variations in shear rate, amount of calcium and alginate molecular weight were investigated by rheological techniques. Compression tests on quiescent gels were also used to further elucidate the binding mechanism of Calcium to alginate, and the consequences on particle elasticity. This allowed the material properties of fluid gels to be described in relation to the particle structure and inter-particle interactions.

2. Materials and methods

2.1. Materials and sample preparation

Medium viscosity sodium alginate (W201502), CaCO_3 (C6763) and glucono- δ -lactone (G2164) were purchased from Sigma–Aldrich (Sigma–Aldrich Company Ltd., Dorset, UK) and were used without further purification.

Two sodium alginate samples having different molecular weight were also purchased from Sigma–Aldrich (Sigma–Aldrich Company Ltd., Dorset, UK). These two polymers are extracted from the alga *Macrocystis pyrifera* which has an M/G ratio of 1.6, obtained from FTIR spectroscopic analysis (Gómez-Ordóñez & Rupérez, 2011; Mackie, 1971).

The weight average molecular weight (M_w) was reported to be $1.43 \times 10^5 \text{ g mol}^{-1}$ for low viscosity alginate (A2158) and $3.5 \times 10^5 \text{ g mol}^{-1}$ for medium viscosity alginate (A2033) (Pamies, Schmidt, Martínez, & Torre, 2010).

Alginate solutions were prepared by dispersing the biopolymer powder in double distilled water and then stirred while heating to 90 °C until dissolved. CaCO_3 in powder form was dispersed in alginate solutions and sonicated for 10 min in order to decrease particle size. Freshly made aqueous solutions of a slowly

hydrolyzing glucono- δ -lactone (GDL) were added to the alginate and CaCO_3 mixtures immediately prior to sample loading, resulting in a range of final target concentrations.

The CaCO_3 concentrations for 0.5% (w/v), 1% (w/v), 1.5% (w/v) and 2% (w/v) alginate systems used in this study were 7.5 mM, 15 mM, 22.5 mM and 30 mM respectively.

2.2. Fluid gel production in a rheometer

A range of fluid gels were produced from the mixtures of alginate, CaCO_3 and GDL in a Kinexus Rheometer (Malvern Instruments Ltd., UK) using a four bladed vane (25 mm diameter) in cup (27 mm diameter) under shear controlled and isothermal ($T = 25 \text{ °C}$) conditions.

This technique allows control of the applied shear rate as well as monitoring of the rheological response during experimentation. The vane geometry was used to avoid slip effects. In addition, the vane geometry allowed relatively large amount of sample to be produced and analyzed repeatedly in subsequent tests.

Shear viscosity was monitored during fluid gel production with data points recorded every 5 s. The onset time of gelation was identified as the time when a minimum of 7 successive data points showed an increase of more than 0.2% in shear viscosity. The completion time was identified as the time when a minimum of 7 successive data points showed less than 0.08% change in shear viscosity.

2.3. Dynamic oscillatory measurements of fluid gels

The structure and particle interactions of the fluid gels were elucidated by dynamic oscillatory measurements performed on a Kinexus Rheometer (Malvern Instruments Ltd., UK) using a cone and plate geometry (4° cone angle, 40 mm diameter).

The evolution of the dynamic storage modulus (G') after production was monitored at a constant stress of 10 mPa and 1 Hz frequency.

The viscoelastic properties of the produced fluid gels were measured by small amplitude oscillatory experiments at a fixed strain of 0.2%, which was within the linear region.

Strain sweep measurements were performed at 1 Hz on fluid gels 48 h after the production process was finished.

2.4. Polarimetry

Measurements of optical rotation were performed using a Gyromat Automatic Polarimeter (Anton Paar, GmbH) with a sample cell of 5 cm path-length.

2.5. Texture analysis of quiescent gels

Compression tests were carried out on quiescent gels using a TA.XT.plus Texture Analyser (Stable Micro Systems Ltd., UK) with a 40 mm diameter cylindrical aluminium probe. All gel cylinders had a height of 10 mm and a diameter of 23.04 mm. The rate of compression was 1 mm s^{-1} for all samples.

Equations (1)–(4) were used to calculate the true stress ε_H and true strain σ_T values from the force/distance data, where H_0 and A_0 are the initial height and cross-sectional area, h and F are the height and the compression force applied, ε_E and ε_H are the engineering and true strain and σ_E and σ_T are the engineering and true stress (Moresi & Bruno, 2007):

$$\sigma_E = F/A_0 \quad (1)$$

$$\varepsilon_E = (H_0 - h)/H_0 \quad (2)$$

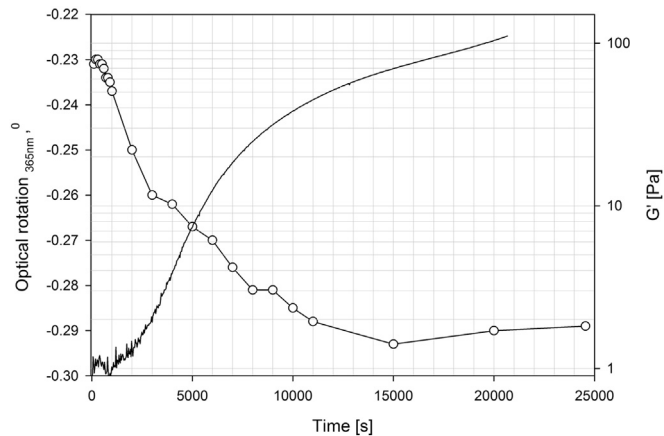


Fig. 1. Optical rotation (○) according to the GDL hydrolysis for 7.5 mM CaCO_3 , 15 mM GDL mixtures. Evolution of G' (black line) for a quiescent gel produced from 0.5% (w/v) alginate, 7.5 mM CaCO_3 , 15 mM GDL.

$$\varepsilon_H = \ln(1 + \varepsilon_E) \quad (3)$$

$$\sigma_T = \sigma_E(1 + \varepsilon_E) \quad (4)$$

The Young's modulus (E) was calculated as the slope of the initial linear region of the true stress/true strain curves as described elsewhere (Norton, Cox, & Spyropoulos, 2011).

3. Results and discussions

3.1. Kinetics of alginate fluid gel formation

3.1.1. Applied shear rate dependence

The internal gelation technique was utilized throughout this work, where a stoichiometric equivalent of glucono- δ -lactone (GDL) was used to ensure that a pH = 7 was held constant, thus avoiding the formation of alginic acid gels. Evidence for the GDL hydrolysis was obtained by polarimetry. Fig. 1 shows the change in optical rotation of linearly polarized light induced by transition from GDL to a mixture of δ -lactone, γ -lactone and gluconic acid as a 365 nm light beam was passed through the CaCO_3 –GDL system.

The release of protons from the GDL hydrolysis gradually liberates calcium ions from the insoluble CaCO_3 salt in an acid-based reaction. The Ca^{2+} ions then trigger the association of the poly-guluronate sequences of the alginate chain by dimerization

mechanism, giving rise to aligned ribbon-like assemblies with cavities into which calcium ions are located (i.e. egg-box dimers) (Grant, Morris, Rees, Smith, & Thom, 1973). As the extent of association increases through aggregation of ordered dimers, clusters expand in size until they form a continuous three-dimensional crosslinked network. This increases the relative contribution of the storage modulus, G' (Fig. 1).

The progressive introduction of calcium ions to the aqueous phase allows uniform distribution of ions throughout the system which will prevent large clusters and will minimize the formation of inhomogeneous gelled networks when producing fluid gels.

The shear required to form fluid gels was provided by the rheometer, where the instantaneous viscosity was measured as a function of time for a range of applied shear rates. As shown in Fig. 2, all solutions displayed virtually Newtonian behaviour before the onset time of gelation. This is ascribed to the binding of calcium to a single guluronate unit that occurs under limited calcium conditions, prior to the formation of egg-box dimers. As a result, monocomplexes are formed, decreasing the charge density on the polymer chain and giving rise to local charge reversal (Fang et al., 2007; Siew, Williams, & Young, 2005). These monocomplexes are well separated from each other, hence remaining relatively insensitive to the shear. For high M_w alginates, the formation of such compact structures resulted in a significant decrease of viscosity, as will be further discussed later in this paper.

As more calcium ions become available, the pair wise association between monocomplexes forms one-dimensional egg-box dimer structures which then aggregate via inter-cluster associations in a process that occurs simultaneously with the shear induced break-up of the network being formed. As a consequence of these competing mechanisms, microgelled particles are formed.

Norton et al. (1999) proposed a nucleation and growth mechanism for fluid gel particle formation, where the initial clusters (i.e. gel nuclei) can grow through enrichment from the non-gelled continuous phase or via coalescence/agglomeration of those gel nuclei.

Assuming that gel nuclei form at the beginning of the kinetic experiment, their initial volume fraction would not be great enough as to display any crowding effect and the viscosity would remain Newtonian, as it is shown in Fig. 2. The subsequent increase in viscosity is attributed to a simultaneous increase in number and volume fraction of particles (Norton et al., 1999).

The time at which this process starts (onset time of gelation) increases as the magnitude of the applied shear force is increased (Fig. 2, Table 1) which indicates a reduction in the rate of particle growth. This suggests that the applied shear rate limits the

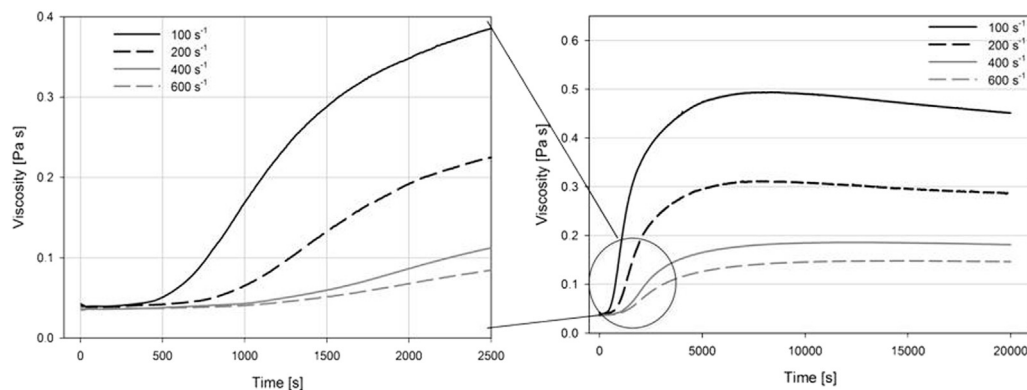


Fig. 2. Viscosity during fluid gel production as a function of time. Systems were produced at a range of applied shear rates from 1.5% (w/v) alginate, CaCO_3 22.5 mM, 45 mM GDL and isothermally at 25 °C.

Table 1

Onset and completion times of gelation for alginate fluid gels produced at a range of shear rates. All systems were prepared from 1.5% (w/v) alginate, 22.5 mM CaCO₃ and 45 mM GDL.

Shear rate [1/s]	Onset		Completion	
	T_0 [s]	η_0 [Pa s]	T_c [s]	η_c [Pa s]
100	405	0.044	6690	0.492
200	660	0.045	6655	0.309
400	1000	0.043	6540	0.176
600	1175	0.043	6410	0.136

likelihood of an association event to occur between the early formed particles. In addition, these results represent the first experimental evidence for the shear rate dependence of the rate of particle growth. Further data supporting this view are provided later in this study.

Viscosity change ($\Delta\eta$) during formation as a function of the applied shear rate ($\dot{\gamma}$) is presented on a double logarithmic plot in Fig. 3. The data was fitted to the power law equation previously used with agarose and k-carrageenan sheared gels (Emanuele & Palma-Vittorelli, 1992; Gabriele, Spyropoulos, & Norton, 2009):

$$\Delta\eta = B(\dot{\gamma})^{-C} \quad (5)$$

As shown in Table 2, both parameters B and C increase with increasing polymer concentration. For fluid gels produced with same molar ratio calcium to alginate, the following empirical equation describing the increase of B with polymer concentration was found, where C_{alg} is alginate concentration:

$$B = 9.99C_{\text{alg}}^{2.24} \quad (6)$$

The effect of the applied shear rate was further explored by performing frequency sweeps on the fluid gels 48 h after production. Fluid gels produced at the lowest shear rate (50 s⁻¹) displayed the highest G' values as one would expect due to increased inter-particle contact of a suspension with large particles and a high particulate phase volume (Fig. 4). An increase in particle size for fluid gels produced with lower shear rates has been reported in previous works on agar and k-carrageenan fluid gels (Gabriele et al., 2009; Norton et al., 1999). Thus, throughout this work, shear rates above 50 s⁻¹ were employed so as to prevent the formation of large aggregates of particles.

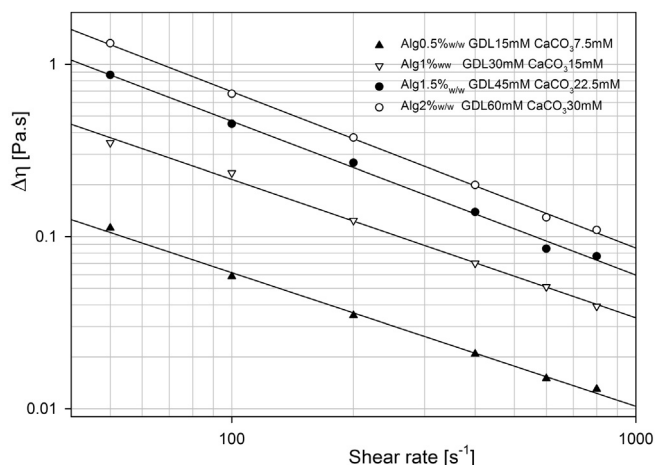


Fig. 3. Viscosity change as a function of applied shear rate during formation of alginate fluid gels. Data is presented in a double logarithmic plot.

Table 2

Parameters of the power law equation (5) describing the dependence of viscosity increase on applied shear rate for fluid gels produced at a range of alginate concentrations.

Parameters	B	C	R^2
Alg0.5%, GDL 15 mM, CaCO ₃ 7.5 mM	2.19	0.776	0.997
Alg1%, GDL 30 mM, CaCO ₃ 15 mM	8.71	0.804	0.996
Alg1.5%, GDL 45 mM, CaCO ₃ 22.5 mM	28.51	0.893	0.996
Alg2%, GDL 60 mM, CaCO ₃ 30 mM	45.53	0.908	0.998

In contrast to the system produced at 50 s⁻¹, the effect on storage modulus was reduced when 100 s⁻¹ and 800 s⁻¹ shear rate were employed during sheared gel production. Furthermore, the small differences measured in G' were not mirrored by measurements of shear viscometry, as all systems exhibited virtually identical viscosities across all shear rates (Fig. 5). This suggests a comparable elasticity/deformability of particles which in turn determines the flow of these particles past one another.

The reason for this is that fluid gel system, once formed, continues to evolve via inter-particle interaction. It has been argued in the literature that the complete ordering of gels made from aggregating polysaccharide may occur over a time scale of an hour to days (Arnott et al., 1974). Thus, it is expected that the re-ordering process which occurred quiescently (i.e. in the absence of shear) for 48 h led to systems with similar properties. Similarly to agar, it is expected that the applied shear kinetically captures the alginate particle structures into anisotropic morphologies rather than the more thermodynamically favourable spherical shape due to the rapid coil-dimer transitions. These anisotropic shapes favour inter-particle interaction, allowing the material to form a percolated network, thus determining the elasticity of the fluid gel system.

As will be demonstrated later in this paper, the quiescent structuring process due to inter-particle interaction is mainly controlled by calcium concentration rather than the magnitude of the applied shear rate.

3.1.2. Effect of calcium concentration and polymer M_w

We have shown that to a certain degree the level of applied shear affects the kinetics of ordering transition in alginate. This conversion rate from disordered polymer coils to a fluid gel structure is expected to strongly depend on calcium concentration as well as the physicochemical properties of the polymer.

In this study, two alginate samples with different molecular weights (M_w) and equivalent fraction of guluronic acids (F_C) were

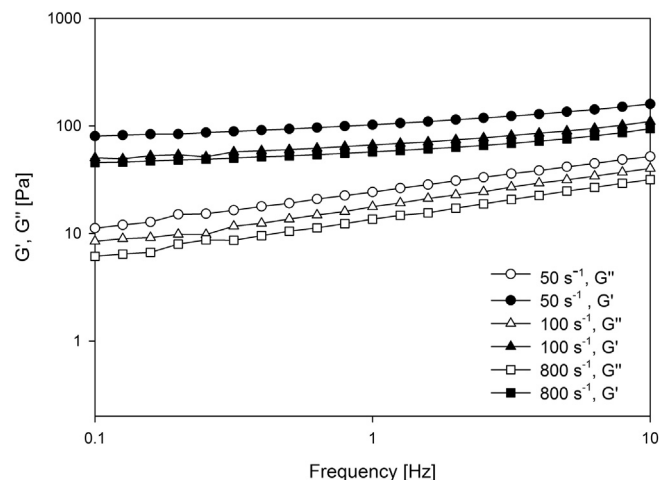


Fig. 4. Mechanical spectra of 1% (w/v) alginate fluid gels produced at different applied shear rates. Tests were conducted at strains within the linear viscoelastic regime.

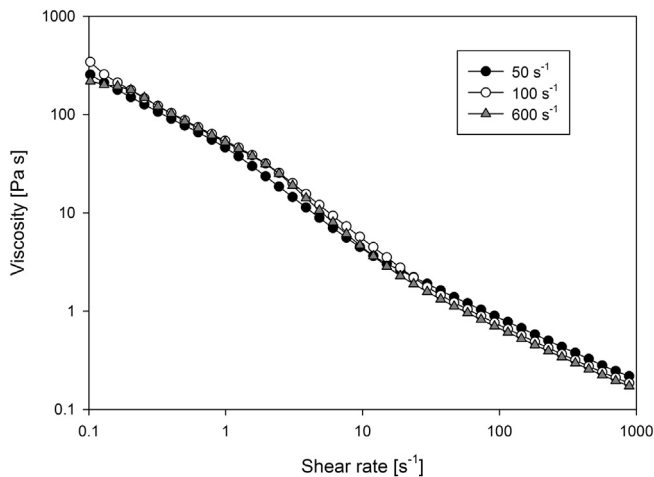


Fig. 5. Flow curves of the alginate fluid gels produced at different shear rates. Measurements were conducted 48 h after the production process.

used to produce fluid gels with a range of CaCO_3 concentrations. Thus, variations in their rheological behaviour could be attributed exclusively to differences in molecular assemblies arising from changes in the number of crosslinks per polymer chain.

From Fig. 6A and B it can be seen that the viscosity decreases prior to the onset time of gelation due to the formation of mono-complexes. This is more significant when high M_W alginates are used, most likely due to the greater flexibility of the long polymer chains, allowing them to contract/shrink to a greater extent.

The data presented in Fig. 6A and B also shows that high M_W alginates have greater viscosities and increased ordering rates compared to the low M_W systems. The higher rate of viscosity increase arises from a greater length scale affected by the same G/Ca interactions compared to the longer molecular weight alginates. This enhances the formation of a percolating network along with an increasing the number of rheologically-effective network crosslinks for the same number of molecular crosslinks at higher polymer molecular weight.

The decreased number of loose chain ends of the high molecular weight alginates could also contribute to this behaviour, probably to a lesser extent, by enabling more calcium ions to be rapidly accommodated between adjacent G-blocks.

This strong influence of polymer M_W is not surprising considering that gelation via in-situ calcium release occurs in a calcium-limited environment and thus the chain characteristics are expected to greatly affect the calcium binding to alginate chain. The calcium binding mechanism and its consequences on the particle's structural properties will be further discussed in Section 3.3.

Within the framework of particle growth occurring by augmentation from the surrounding continuous phase, the faster gelation kinetics measured as a result of increasing calcium concentration is ascribed to an increase of the rate of both coil-dimer transition and dimer-aggregated dimer transition. Even at shear rates as high as 800 s^{-1} , the structuring process occurs more rapidly and to a greater extent when more calcium ions are available. This progressive increase in ordering rate is an indication that the release of calcium is rate limited by the CaCO_3 dissolution rather than the GDL hydrolysis. The reason for this is that the reasonably big CaCO_3 particles ($19 \mu\text{m}$ average particle size) dissolve at a slower rate than the process of GDL hydrolysis. This is in agreement with Ström and Williams (2003) findings which showed that while the H^+ and Ca^{2+} release rates for systems containing $1.7 \mu\text{m}$ CaCO_3 particles and GDL were comparable, the Ca^{2+} release rates of $5 \mu\text{m}$ and $10 \mu\text{m}$ CaCO_3 particles were slower than the H^+ release rate and dependent on CaCO_3 particle size (Ström & Williams, 2003).

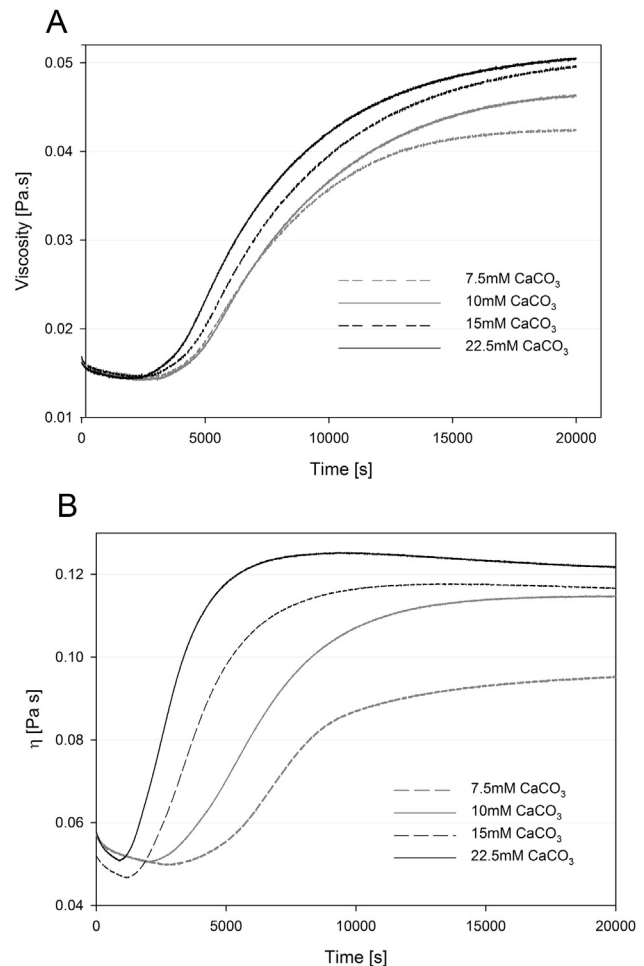


Fig. 6. A. Viscosity curve during formation of low M_W alginate 1% (w/v) fluid gels produced at 800 s^{-1} , 30 mM GDL and at a range of calcium concentrations. B. Viscosity curve during formation of high M_W alginate 1% (w/v) fluid gels produced at 800 s^{-1} , 30 mM GDL and at a range of calcium concentrations.

3.2. Particle growth and inter-particle interactions in the absence of shear

In order to further investigate the effect of processing conditions on particle growth and inter-particle interaction, the dynamic storage modulus was measured at a frequency of 1 Hz and applied stress of 10 mPa after fluid gels were produced. The results obtained for fluid gels produced from 1.5% (w/v) alginate, 22.5 mM CaCO_3 , 45 mM GDL at a range of shear rates are presented in Fig. 7.

The resultant time dependence curve of G' was reduced empirically to the exponential growth equation (Eq (3)) previously used in agarose fluid gels (Gabriele et al., 2009):

$$G'(t) = G'_\infty - (G'_\infty - G'_0) \exp^{-k_i t} \quad (7)$$

where k_i is kinetic constant, G'_0 is the initial storage modulus immediately after stopping the shear and G'_∞ the storage modulus at infinite time. The calculated values are shown in Table 3.

The most interesting result to emerge from this analysis is that the relative rate of ordering k_i remains unaffected by the magnitude of applied shear rate. That is, once the shear is removed, the rate at which the structure is strengthening is independent of the applied mechanical forces. This behaviour occurs when the processing time is greater than the gelation time so that complete molecular

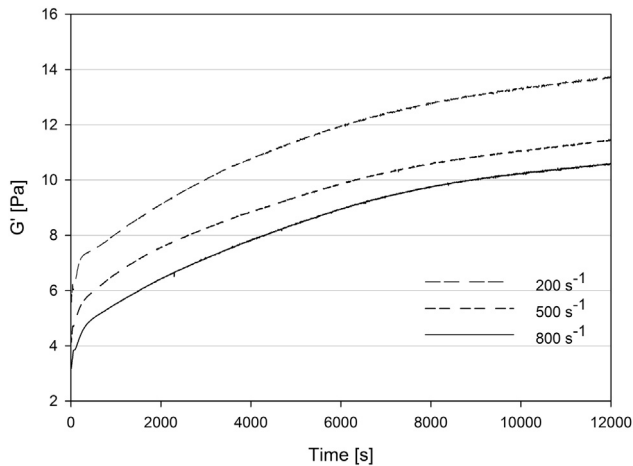


Fig. 7. G' as a function of time and applied shear rate measured after a production process of 6 h. Fluid gels were produced from 1.5% (w/v) alginate, 22.5 mM CaCO_3 , 45 mM GDL.

Table 3

Model parameters from the exponential equation (3) describing the inter-particle interactions after production of fluid gels at different applied shear rates. Concentrations of sodium alginate, CaCO_3 and GDL were fixed at 1.5% (w/v) and 22.5 mM and 45 mM, respectively.

$G'(t) = G'_\infty - (G'_\infty - G'_0) \exp^{-k_i t}$			
SR [s^{-1}] ^a	200	500	800
G'_0 [Pa]	6.72	5.44	4.24
G'_∞ [Pa]	14.73	12.51	11.51
k_i [s^{-1}]	0.0002	0.0002	0.0002
R^2	0.998	0.996	0.998

^a SR [s^{-1}]: applied shear rate.

ordering occurs under shear, thus the ordering process post-production is driven by inter-particle interactions.

A possible explanation for the equal rates of inter-particle interaction is that particles with similar surface properties are formed at the experimental range of shear rates. The proportion of disordered coils at the particle surface that form due to late polymer ordering (Norton et al., 1999) are expected to play a determining role in the extent of inter-particle bridging.

When comparing the evolution of G' for systems produced from different M_W alginates and under the same conditions of shear rate and processing time, different responses were observed: while the behaviour of the high M_W system can be described by the previously proposed exponential grow model, the best fit for the data obtained from low M_W alginates is the following linear function:

$$G'(t) = G'_0 + k_g t \quad (8)$$

Table 4A

Model parameters from the polynomial first order equation (8) calculated by fit to the data of low M_W alginates produced at different CaCO_3 and applied shear rates. The concentrations of alginate and GDL were kept constant at 1% (w/v) and 30 mM, respectively.

Low M_W alginate $G'(t) = G'_0 + k_g t$									
	7.5 mM CaCO_3		10 mM CaCO_3		15 mM CaCO_3		22.5 mM CaCO_3		
SR [s^{-1}] ^a	200	800	200	800	200	800	200	800	
G'_0 [Pa]	1.89	1.24	3.46	2.49	3.65	2.84	4.69	3.07	
k_g [s^{-1}]	0.0003	0.0002	0.0004	0.0003	0.0009	0.0008	0.001	0.0009	
R^2	0.965	0.947	0.971	0.969	0.999	0.993	0.998	0.997	

^a SR [s^{-1}]: applied shear rate.

This result is explained by the fact that the final particulate structure was not completely formed when the shear was removed thus mainly quiescent particle growth drives the evolution of G' over time. This idea is in agreement the previous results which showed lower reaction rates for the low M_W alginates.

As shown in Table 4A, the ordering rates (k_g) increased as the applied shear rate was decreased for all systems. Under these conditions where particle growth rather than particle interaction controls the rate of ordering, these results confirm that particles grow at a faster rate as the magnitude of the applied shear is lowered.

Conversely, the crosslinking process of the high M_W system appeared to have already occurred to its full extent after 4 h of processing. Hence, no particle growth occurred once the shear was removed and the rate of ordering was determined by inter-particle interaction and, consequently, was independent on the shear rate (Table 4B).

This hypothesis is further supported by comparing the effect of calcium concentration on the relative rate of ordering between the low and high M_W systems. The data reveals that when the shear environment is stopped before completion (low M_W alginate), the ordering rate (particle growth) increases significantly with calcium concentration (Table 4A), as would be expected due to an increased rate of coil-dimer transition. Differently, only a minor calcium concentration effect is measured when the ordering rate is driven by inter-particle interaction (Table 4B).

Thus, it is concluded that when the process of particle growth is completed under shear, the rate at which particles interact post-processing is not dependent on applied shear rate but slightly on calcium concentration. When the shear is removed before the gelled particles are completely formed, the rate of particle growth is controlled by both the applied shear rate and calcium concentration.

3.3. Textural properties of alginate fluid gels

As shown in this study, the viscosity increases during formation with increases in calcium concentration. This may be due to the formation of stiffer (less deformable) particles arising from more alginate chains being bound together in junction zones by multiple Ca^{2+} ions which leads to a higher density of crosslinking.

This hypothesis was tested by performing oscillatory strain sweeps at a frequency of 1 Hz on the produced fluid gels 48 h after production. Results showed greater limiting strains for particles produced with reduced calcium concentrations (Fig. 8). That is, the effect of progressively decreasing CaCO_3 concentration from 22.5 mM to 10 mM is to increase the deformability of particles such that they can withstand larger strains before flow occurs.

However, the limiting strain of particles produced with 30 mM CaCO_3 is higher than expected. This abrupt reduction in particle stiffness is attributed to an excess calcium which restrains the packing of chains into perfectly aligned dimers, leading to both inter-chain and intra-chain binding (Smidsrød, 1974). These

Table 4B

Model parameters from the exponential equation (7) calculated by fit to the data of high M_W alginates produced at different CaCO_3 and applied shear rates. The concentrations of alginate and GDL were kept constant at 1% (w/v) and 30 mM, respectively.

High M_W alginate $G'(t) = G'_\infty - (G'_\infty - G'_0)\exp^{-k_f t}$								
	7.5 mM CaCO_3		10 mM CaCO_3		15 mM CaCO_3		22.5 mM CaCO_3	
SR [s^{-1}] ^a	200	800	200	800	200	800	200	800
G'_0 [Pa]	5.51	2.95	8.54	5.01	12.82	10.14	13.34	12.82
G'_∞ [Pa]	11.42	7.77	13.89	10.89	23.51	21.13	24.32	23.51
k_f [s^{-1}]	0.0001	0.0001	0.0001	0.0001	0.0002	0.0002	0.0002	0.0002
R^2	0.996	0.998	0.991	0.996	0.998	0.995	0.942	0.998

^a SR [s^{-1}]: applied shear rate.

heterogeneous binding sites create junctions between chains that are kinetically more stable towards dissociation.

Thus, it appears to be a saturated CaCO_3 concentration above which the formation of egg-box dimers and hence particle gelled network is disrupted. For the 1% low M_W alginate fluid gel, this critical CaCO_3 concentration was 22.5 mM.

To gain further insight into the crosslinking mechanism, the strengths of quiescent gels formed at a range of calcium concentrations were measured. These results are presented in Fig. 9, where the elastic Young's modulus (E) was determined as the slope of the initial linear region of true strain/true stress diagram (details on this analysis are provided in the Methodology section).

A steady increase of the Young's modulus is seen between 10 mM and 22.5 mM CaCO_3 which is followed by a sharp increase with the increased degree of crosslinking from 22.5 mM up to 45 mM CaCO_3 (Fig. 9). These results are consistent with those of Moe, Draget, Skjåk-Bræk, and Simdsrød (1992) who argued that calcium ions, after being liberated from the CaCO_3 , are accommodated within the longest junction zones before creating new ones. The general view is that G-blocks dimerize in such a way that the alginate chains are bound together by multiple cations in long junction zones rather than distinct, point-like crosslinks (Grant et al., 1973; Stokke, Smidsrød, Bruheim, & Skjaak-Braek, 1991).

From Fig. 9 it can also be seen that decline in gel strength occurs for concentrations above 45 mM CaCO_3 which, for the 2% (w/v) alginate quiescent gel, corresponds to the same critical molar ratio CaCO_3 :alginate that has been shown earlier for fluid gels.

Assuming that the gel strength is determined by the number of crosslinks per unit volume (Stokke et al., 1991), the fact that both fluid and quiescent gels yield exhibit the same critical molar ratio CaCO_3 :alginate suggests that similar degree of crosslinking

occurs in both sheared and quiescent gelation. Hence, the polymeric gelled network within an individual fluid gel particle is equivalent to that of their counterpart quiescent gel. This is likely to be due to the almost instantaneous coil-dimer transition of alginate in the presence of calcium which, under the imposed shear field, leads to kinetically trapped dimer structures. These findings are consistent with those of Frith, Garijo, Foster, and Norton (2002) who demonstrated that the elasticity of individual agarose fluid gel particles is the same as that of their equivalent quiescent gel. Similarly to alginates, the coil-helix transition of agarose occurs on a millisecond time scale (Norton, Goodall, Austen, Morris, & Rees, 1986), so it seems that the potential to form kinetically trapped fluid gel structures is a common feature of aggregating polysaccharides with fast molecular ordering transitions.

Nonetheless, the response of alginate fluid gels appears to differ from their corresponding quiescent gels with respect to the calcium concentration effect on gel strength. The dynamic storage modulus (G') of fluid gels showed an initial abrupt rise as the crosslink density increased with increasing CaCO_3 concentration from 10 mM to 22.5 mM, followed by a moderate increase from 22.5 mM to 45 mM (Fig. 10).

In contrast to quiescent gels whose elasticity is only determined by the mechanism of calcium chelation into junction zones, the formation of percolated networks due to particle interactions is influenced to a greater extent by the particle surface (e.g. presence of disordered coils) than the calcium concentration. Thus, in comparison to the Young's elastic modulus (E) of quiescent gels, the G' of fluid gels has a lower dependence on CaCO_3 concentration between 22.5 mM and 45 mM.

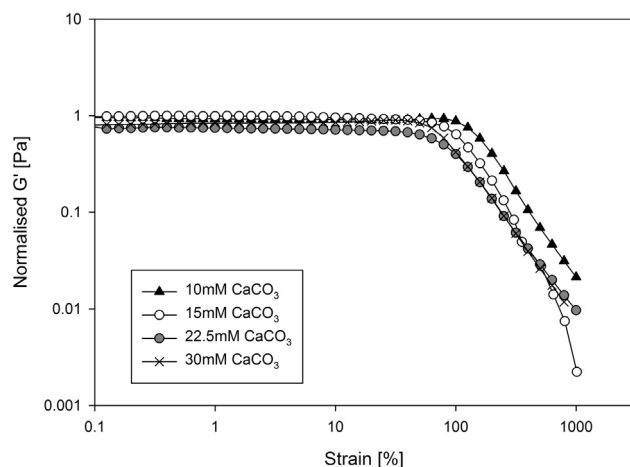


Fig. 8. Normalized storage modulus versus strain of alginate 1% (w/v) fluid gels produced at a range of CaCO_3 concentration.

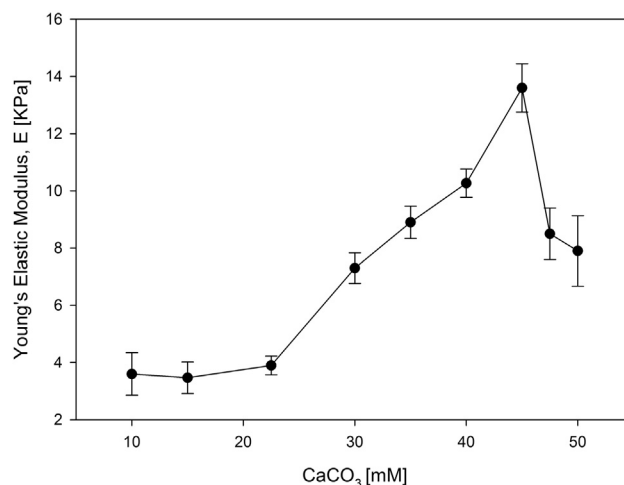


Fig. 9. Young's elastic modulus as function of CaCO_3 concentration for 2% (w/v) low M_W alginates quiescent gels. The GDL concentration was kept constant at 30 mM for all systems.

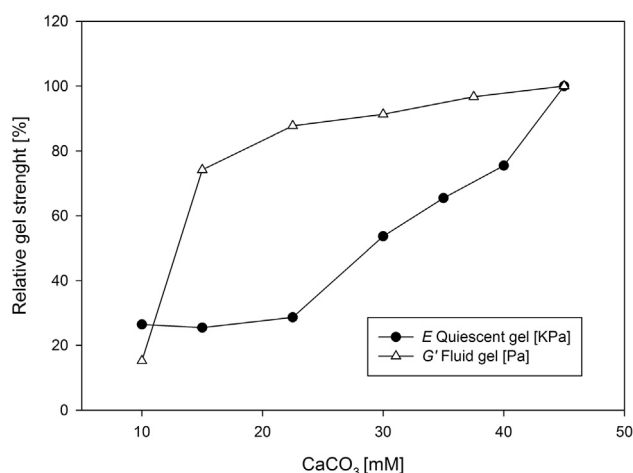


Fig. 10. Young's modulus and G' as function of CaCO_3 concentration for quiescent and fluid gels, respectively. Relative data is calculated by setting the highest strength value to 100%.

4. Conclusions

The kinetics of alginate fluid gel formation has been studied using in-situ calcium release and under controlled shear conditions. The negative correlation between the viscosity increase and the applied shear rate during production was described using a power law equation.

Rheological results showed that the onset time of gelation increased with increasing the applied shear rate which suggests faster rates of particle growth. These results were confirmed by measurements of G' when shear was removed before completion of fluid gel particle formation. The rate of particle growth increased significantly as a result of either reducing the level of shear or increasing calcium concentration.

On the contrary, when the shear was removed after completion of the fluid gel structure, the rate of re-ordering due to inter-particle interaction appeared not to be affected by the magnitude of applied shear.

Changes in the gelation kinetics and rheological response of fluid gels produced with alginates having different M_w confirmed the significant influence of the chain length and fraction of polymer loose-ends on the degree of crosslinking. High M_w alginate fluid gels exhibited faster formation kinetics, enhanced viscosities and greater G' than those of fluid gels produced with low M_w alginate.

Similarly, increasing calcium concentration increased fluid gel particle stiffness such that lower limiting strains were measured. A saturated calcium concentration, corresponding to a critical CaCO_3 :alginate molar ratio, was identified for both quiescent and fluid gel systems. This suggested that sheared and quiescent gels have similar network connectivity and equivalent numbers of polysaccharides arranged in egg-box dimers, probably due to the fast dimerization process which kinetically captures the fluid gel structure.

With regard to the calcium concentration effect on the gel strength, the fluid gel's response appeared to be different to that of a quiescent gel at CaCO_3 below the critical value. The distinct textural response of fluid gels is ascribed to the interaction between particles that enables an elastic network to form with less dependence on calcium concentration than seen in quiescent gels.

Overall, this work has shown that the kinetics of fluid gel formation and the resulting particle properties can be manipulated by

the alginate M_w and calcium concentration, even using the same Guluronic/Mannuronic block composition. Thus, the ability to control them can ultimately allow the design of alginate fluid gels with specific textural properties.

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